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Antibacterial properties of tannins isolated from leaves and fruits of *Emblica officinalis* Gaertn.

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ABSTRACT

The tannins isolated from *Emblica officinalis* were screened against *Escherchia coli, Pseudomonas aeruginosa, Bacillus subtilis, Shigella boydii, Shigella flexneri, Staphylococcus aureus* and *Staphylococcus epidermidis* using agar well diffusion method. The tannins showed antibacterial properties against all the test bacteria. The tannins extracted from leaves were more antibacterial than the tannins from fruits. Out of all tested bacteria *E.coli* was more inhibited. The minimum inhibitory concentration of the tannins ranged between 5 to 8 mg/ml.

Key Words: Antibacterial, agar well diffusion, tannins.

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INTRODUCTION

Tannin is general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution. Tannins can be toxic to bacteria, fungi and yeast [1]. Tannins have been isolated from some plants and they exhibited antibacterial properties [2]. *E. officinalis* and *Coriandrun sativum* are inhibitory to gram negative bacteria [3]. The *Emblica officinalis, Terminalia chebula*, and *T. bellerica* have antimicrobial properties and inhibited the growth of *Streptococcus* mutans and gram positive cocci [4]. The species of Combretaceae showed antibacterial properties because of the presence of tannins [5].

Emblica officinalis with antibacterial property is being used extensively in Indian system of medicine [6]. *Embilica officinalis* was screened for its potential antibacterial activity against pathogenic bacterial strains. The fruit of *E. officinalis* is commonly known as amla and is highly valued in tradional Indian medicine [7]. Several constituents of *E. officinalis* fruits have been identified, mainly the hydrolysable tannins, emblicanin A, emblicanin B, pnigluconin and pedunculagin [8]. Emblicanin A and B have been proposed to be the active constituents with significant *in vitro* antimicrobial properties [9].

MATERIALS AND METHODS

Collection of plant materials

The leaves and fruits of test plant *Emblica officinalis* were collected from Nanded region of Maharashtra state.

Source of microorganisms

The bacteria selected for study were common human pathogens like *E. coli* (ATCC-10412), *Pseudomonas aeruginosa* (ATCC-27853), *Bacillus subtilis, Shigella boydii, Shigella Flexneri, Staphylococcus aureus* (ATCC-103207) and *Staphylococcus epidermidis.* They were obtained from Microbiology Department of Yeshwant Mahavidyala, Nanded, (M.S.).

Standardization of microorganisms

For standardization of microorganism exactly 0.5 ml of overnight cultures of each organism was dispended into 20 ml of sterile nutrient broth and incubated for 3 hours to standardize the culture to 10^6 cfu/ml. A loop-ful of the standard culture was used for the antibacterial activity [10].

Extraction of tannins

The powdered leaves and fruit samples (5g) were boiled in 10 ml of distilled water for 3 minutes on a hot plate. The mixture was filtered while hot and the resulting filtrate was used to carry out ferric chloride test. Sample of the filtrate (1 gm) was weighed in to a beaker and 10 ml of distilled water added. This was boiled for 5 minutes. Two drops of 5%

ferric chloride ($FeCl_2$) was than added. Production of greenish precipitate indicated the presence of tannins.

Assessment of antibacterial activity of plant extracts

Antibacterial activity test was evaluated by Agar well-diffusion method and expressed by diameter zone of inhibition in mm. The bioassay was carried out by using 1 ml of inoculum prepared from an overnight culture for given test bacterium, 1 ml of the bacterial cell suspension was poured in the Petri plate and the plates were poured with respective medium. The medium was allowed to solidify and wells were prepared using sterilized cork borer (diameter 5 mm). The cuts agar disks were carefully removed by the use of forceps sterilized by flaming. Each well was filled with different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 etc. mg/ml) of tannins isolated from the plant extract. Plates were then incubated aerobically at 28 ± 2^{o}C for 24 hrs. The experiments were conducted with three replications. The zones of inhibitions were then recorded.

Determination of minimum inhibitory concentration

Isolated tannins from *E. officinalis* were used to check MIC. Different concentrations of tannins ranging between 5 to 8 mg/ml were introduced with an overnight culture of *E.coli, Pseudomonas aeruginosa, Bacillus subtilis, Shigella boydii, Shigella flexneri, Staphylococcus aureus* and *Staphylococcus epidermidis* diluted to give a final concentration of 10^6 cfu/ml. The tubes were incubated at 28 ±2^{o}C for 24 hrs. The least concentration of tannin that did not permit any visible growth of the inoculated test bacteria in broth culture was regarded as minimum inhibitory concentration (MIC) in each case [10].

RESULTS AND DISCUSSION

The isolated tannins from leaves and fruits of *E. officinalis* in this study showed antibacterial activity against *E.coli, P. aeruginosa, B. subtilis, S. boydii, S. flexneri, S. aureus* and *S. epidermidi*s (Table 1 and 2). The tannins isolated from leaves exhibited maximum antibacterial activity than fruits tannins. The antibacterial activity increases with increase in concentration of tannins isolated in this study is similar with previous work [11]. They reported that as the concentration increases, the antibacterial activity also increases. Out of all bacteria tested in this study *E. coli* showed maximum inhibition in both tannins isolated from leaves and fruits while *S. epidermidis* showed least inhibition. The *E. officinalis* plant which is rich in tannins has been shown to possess antimicrobial properties against test microorganisms this study is agreement with previous work [12-14]. The antimicrobial activity of plants is observed due to tannins, alkaloids and glycosides [15].

The minimum inhibitory concentration (MIC) of tannins isolated from leaves and fruits in this study against the test bacteria ranged between 5 to 8 mg/ml (Table 3). Antimicrobial agents with low activity against an organism have a high MIC while a high active antimicrobial agent gives a low MIC.

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Table 1. Antibacterial effects of tannin isolated from leaves of *E. officinalis*.

Conc. (mg/ml)	Ec	Pa	Bs	Sb	Sf	Sa	Se
0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	3.2	2.1	3.5	4.2	2.5	3.1	3.6
1.5	8.5	8.3	7.2	8.5	5.2	6.3	6.0
2.0	9.5	9.0	8.3	8.7	7.9	6.9	8.0
2.5	10.7	10.5	10.5	10.3	9.0	8.2	8.5
3.0	11.5	11.3	11.2	10.9	9.5	8.8	8.5
3.5	12.9	11.8	11.9	11.2	10.5	9.0	9.5
4.0	15.2	13.8	12.8	13.5	11.5	9.3	10.9
4.5	17.9	16.5	15.8	15.3	13.4	13.7	12.2
5.0	18.0	17.1	16.3	16.2	13.5	13.9	12.2

0.0- Negligible. Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis*, Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Sa-*Staphylococcus aureus* and Se-*Staphylococcus epidermidis.*

Table 2. Antibacterial effects of tannin isolated from fruits of *E. officinalis*.

Conc. (mg/ml)	Ec	Pa	Bs	Sb	Sf	Sa	Se
0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.5	2.2	1.2	2.0	1.3	0.0	0.0	1.5
2.0	3.1	2.5	2.8	2.9	1.5	2.0	1.8
2.5	5.3	3.2	4.2	3.0	3.9	2.9	2.9
3.0	6.2	4.9	5.0	4.8	4.7	2.9	3.8
3.5	6.8	6.0	6.0	5.2	5.1	4.0	4.2
4.0	6.9	6.2	6.2	8.3	5.9	4.8	5.2
4.5	8.0	7.7	7.3	8.3	7.0	5.0	5.29
5.0	10.1	7.8	8.8	8.3	8.1	6.9	6.8

0.0- Negligible. Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis,* Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Se-*Staphyolcoccus aureus* and Se-*Staphylococcus epidermidis.*

Table 3. Minimum inhibitory concentration (MIC) of tannins isolated from leaves and fruits of *E. officinalis.*

Organism	MIC (mg/ml) of leaf tannins	MIC (mg/ml) of fruit tannins
Ec	5.0	6.5
Pa	5.5	7.0
Bs	5.5	7.0
Sb	6.0	7.5
Sf	7.5	8.0
Sa	8.0	8.0
Se	0.8	0.8

Ec-*E. coli*, Pa-*Pseudomonas aeruginosa*, Bs-*Bacillus subtilis*, Sb-*Shigella boydii*, Sf-*Shigella flexneri*, Sa-*Staphylococcus aureus* and Se-*Staphylococcus epidermidis*.

CONCLUSION

The result of the present study suggest that the tannins isolated from leaves and fruits of *E. officinalis* possessed remarkable antibacterial activity. Thus there is possibility of developing the plant as a source of antimicrobial agent.

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